Different compositions of pharmaceuticals in Dutch and Belgian rivers explained by consumption patterns and treatment efficiency

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Abstract

In the current study 43 pharmaceuticals and 18 transformation products were studied in the river Meuse at the Belgian-Dutch border and four tributaries of the river Meuse in the southern part of the Netherlands. The tributaries originate from Belgian, Dutch and mixed Dutch and Belgian catchments. In total, 24 pharmaceuticals and 13 transformation products were observed in samples of river water collected from these rivers. Observed summed concentrations of pharmaceuticals and transformation products in river water ranged from 3.5 to 37.8 µg/L. Metformin and its transformation product guanylurea contributed with 53 to 80 % to this concentration, illustrating its importance on a mass basis. Data on the flow rate of different rivers and demographics of the catchments enabled us to calculate daily per capita loads of pharmaceuticals and transformation products. These loads were linked to sales data of pharmaceuticals in the catchment. Simple mass balance modelling accounting for human excretion and removal by sewage treatment plants revealed that sales could predict actual loads within a factor three for most pharmaceuticals. Rivers that originated from Belgian and mixed Dutch and Belgian catchments revealed significantly higher per capita loads of pharmaceuticals (16.0 ± 2.3 and 15.7 ± 2.1 mg/inhabitant/day, respectively) than the Dutch catchment (8.7 ± 1.8 mg/inhabitant/day). Furthermore, the guanylurea /metformin ratio was significantly lower in waters originating from Belgium (and France) than in those from the Netherlands, illustrating that sewage treatment in the Belgian catchment is less efficient in transforming metformin into guanylurea. In summary, the current study shows that consumption based modeling is suitable to predict environmental loads and concentrations. Furthermore, different consumption patterns and wastewater treatment efficiency are clearly reflected in the occurrence and loads of pharmaceuticals in regional rivers.

Introduction

Numerous studies describe the presence of pharmaceuticals in the aqueous environment (Monteiro and Boxall, 2010; Roig, 2010). These pharmaceuticals can originate from human consumption, veterinary consumption, aquaculture or (accidental) emissions during production (Larsson et al., 2007) and transport of pharmaceuticals. Consumed human pharmaceuticals are excreted by humans via urine and feces. They are subsequently transported to sewage treatment facilities and are (partially) emitted to surface waters. However not all consumed pharmaceuticals end up in the environment since they can be metabolized in the human body . After excretion, pharmaceuticals and metabolites can be further transformed or sorbed in sewage systems, sewage treatment plants (active sludge) and receiving waters. The rates and nature of the metabolism, transformation and sorption depend on the chemical properties of the pharmaceutical the physiology of the person using the medication and environmental conditions in wastewater treatment and receiving waters. While parent pharmaceuticals have been studied intensively in the environmental over the last decades metabolites and transformation products (later all referred to as transformation products) have drawn less attention in monitoring and risk assessment (Escher and Fenner, 2011).

Databases on the sales of pharmaceuticals and extensive knowledge of excretion and metabolism, stability and sorption in commonly applied sewage treatment systems, and stability and sorption in receiving waters enables one to relate sales to loads in surface waters (Castiglioni et al., 2004; Liebig et al., 2006; Alder et al., 2010; ter Laak et al., 2010; Kugathas et al., 2012). Furthermore, pharmaceutical consumption varies between regions (Van den Berg Jeths and Van Batenburg-Eddes, 2003) and countries (Mossialos et al., 2004), while sewage treatment practice and environmental conditions differ between regions, countries (Boyjoo et al., 2013) and seasons (Sui et al., 2011). Regional consumption data and knowledge on sewage treatment practice can guide monitoring efforts in different regions, countries or seasons and define ‘hot spots’ where environmental risks are the highest. Furthermore, it

65 can be applied to model regional emissions and predict emissions under different consumption-, waste
66 water treatment- and river discharge scenarios.
67
68 The assessment of water quality for the ecosystem health and for drinking water production, can
69 benefit from knowledge of regional sources, emissions and occurrence of pharmaceuticals.
70 Additionally, it can benefit from knowledge on the formation and occurrence of transformation
71 products. In the present work, three approaches are defined to gain knowledge and assess the fate and
72 behaviour of pharmaceuticals and transformation products in the environment.
73
74 First, ratios of pharmaceuticals and transformation products were studied in order to reveal whether
75 ratios between parent compounds and their products are stable and might allow prediction of one
76 concentration from the other. Ratios between different pharmaceuticals were not studied, but this
77 might be of interest in future research. Secondly, the occurrence of pharmaceuticals and
78 transformation products was studied and linked to available sales data to reveal whether sales data are
79 suitable to predict loads in regional surface waters. Finally, the composition of pharmaceuticals in
80 different rivers fed by sewage from Belgium and the Netherlands were qualitatively and quantitatively
81 evaluated to reveal differences between countries.
82

Material and method

Sampling locations

Samples were taken in the river Meuse catchment of the province of Limburg, the most southern province of the Netherlands (Figure 1). Water from the river Meuse was sampled near Eijsden where the river Meuse crosses the border between Belgium and the Netherlands. Four tributaries of the river Meuse i.e. the river Jeker, the river Geul and the river Geleenbeek and river Slijbeek (later referred to as the Jeker, Geul, Geleenbeek and Slijbeek) were sampled near the point where they flow into the river Meuse or into a channel that is connected to the river Meuse (i.e. Slijbeek).

FIGURE 1

Table 1 shows the median, minimum and maximum flow rates of the selected rivers. Furthermore, the number of sewage treatment plants discharging to the different waters and the number inhabitants of the catchments are listed. These data are used to predict loads of pharmaceuticals in the different waters and relate predicted loads to monitoring data.

TABLE 1

Each of the five locations was sampled in 2011 on November the 23rd, November the 30th, December the 7th and December the 12th 2011 between 9:00 and 13:00 by grab sampling. Water was collected in 1000 mL ultra-clean dark green glass bottles and directly stored at 4°C for a maximum of 4 weeks. Further details can be found in Table S1, of Supplementary Information. Grab sampling was used because time integrated sampling was not feasible at the sampling locations.

Pharmaceuticals and transformation products

Samples were analysed for 43 pharmaceuticals and 18 transformation products. The pharmaceuticals were selected based on consumption, occurrence in the environment, physicochemical properties, their availability as standards and whether they can be analysed (de Voogt et al., 2009). Pharmaceuticals and transformation products were obtained from Sigma Aldrich, AK Scientific, Frinton Laboratories and CHEMOS. The purity of the chemicals was > 97 %, except for clindamycin (88.3 %), erythromycin (95 %) and N-acetyl-4-aminoantipyrine (97 %). Deuterated (internal) standards (Atenolol-d7, Atrazine-d5, Bentazone-d6, Benzotriazool-d4, Carbamazepine-d10, Fluoxetine-d5, Gemfibrozil-d6, Metformin-d6, Paracetamol-d3, Phenazone-d3 and Sulfamethoxazole-d4) were obtained from CDN Isotopes. Detailed information on the pharmaceuticals studied is listed in Table S2 of the Supplementary Information.

Analysis of pharmaceuticals and transformation products in water

Aqueous samples were filtered over a 0.20 µm regenerated cellulose filter (Whatman, Spartan 30/B). 100 µL of the filtrated water samples (spiked with 0.5 µg/L internal standards) were directly injected on a Thermo Scientific TSQ Vantage LC-MS/MS equipped with an Accela autosampler and pump. The LC system was equipped with either a reversed phase column, *i.e.* a 100x2.1 mm Hypersil Gold column with a particle size of 1.9 µm (Thermo Scientific) or with a ZIC HILIC column, i.e. a 100x2.1mm ZIC-HILIC column with 3.5 µm particle size. The temperature of the columns was held constant at 25 °C. An ESI interface was used to ionize the compounds and analysis was performed both in positive and negative ionization mode. All pharmaceuticals were analyzed using reversed phase chromatography, except for metformin and guanylurea which were analyzed using the HILIC column. Details on LC conditions and gradients are listed in the "Description of chemical analysis" of the Supplementary Information. The ratio of the responses of at least two mass transitions were used for positive identification of the individual compounds. The average response of these two masses was used for quantification and comparison with external calibration standards (Mezcua et al., 2009). All analysis were performed within one

analytical run and blanks and external standards were added every ten samples to monitor analytical performance.

Limits of quantification were generally 0.01 µg/L, except for 4-acetaminophen sulphate, cortisol, cortisone and erythromicine A (all 0.025 µg/L), O-desmethyl naproxen, paroxetine, prednisolone, metformin and guanylurea (all 0.050 µg/L), norfluoxetine, hydroxy ibuprofen and ciprofloxacin (all 0.50 µg/L) and salicylic acid (> 5.0 µg/L) . No corrections were made for recoveries as most recoveries of detected compounds were between 80 and 120 %. Details can be found in Table S2 of the Supplementary Information.

Calculating emissions from monitoring data

Flow rate data with a 15 min (Jeker, Geul, Geleenbeek) or 1 h (Meuse) interval were obtained from regional water authorities. Flow rates showed some scatter in time, therefore, the total water flow over a period of 24h (q_{day}) at the sampling date was multiplied with the observed concentration of the pharmaceutical or TP ($C_{pharmaceutical}$) to calculate the daily load (Q_{day}):

$$Q_{day} = C_{pharmceutical} \cdot q_{day} \qquad \text{eq.1}$$

Furthermore, loads per inhabitant (Q_{inhab}) were calculated to compare emissions of inhabitants from different regions according to:

$$Q_{inhab} = \frac{Q_{day}}{n_{inhab}} \qquad \text{eq. 2}$$

For the Slijbeek no flow rate data were available, so loads could not be calculated.

Predicting emissions from sales data

Sales data of pharmaceuticals in 2011 were obtained from the Dutch Foundation for Pharmaceutical Statistics (SFK) and the Belgian National Institute for Disease- and Disability-Insurance (RIZIV). It was assumed that the consumption of the inhabitants of the Geleenbeek and Geul catchment corresponded

159 to the Dutch and combined Dutch and Belgian national average, respectively. This assumption seems
160 valid since the sales of pharmaceuticals in the region that covers the Geleenbeek basin and the Dutch
161 part of the Geul basin is near 100 % (106 %) of the Dutch average (van Batenburg-Eddes et al., 2002).
162 For the Jeker catchment, regional sales data of 91,645 out of the 101,535 (Directorate General Statistics
163 and Economic Information, 2013) inhabitants in 2011 were obtained. Here it was assumed that the
164 consumption of the 10 % missing inhabitants was the same as for inhabitants of which sales data were
165 obtained.
166
167 Finally, for all catchments, it was assumed that all residents of the catchment discharge all their sewage
168 via sewage treatment plants in their catchment of residence. This assumption might not always be
169 correct since commuting, traveling (holidays) the use of septic tanks and emissions of untreated sewage
170 can bias the actual discharge. There are however no suitable data to correct for these biases.
171
172 Daily emitted loads ($Q_{day\text{-}pred}$) were estimated using respective annual sales data (Q_{year}) of 2011, the
173 fraction that passes the Sewage Treatment Plant (f_{STP}) and the excretion rate by humans (fraction of
174 consumption excreted, f_{Excr}) according to the following equation:

175 $$Q_{day-pred} = \frac{Q_{year}}{365} \cdot f_{Excr} \cdot f_{STP} \qquad \text{eq.3}$$

176 The STP removal rates and human excretion rates are listed in Table 2 en the Dutch and Belgian Q_{year}-
177 values are listed in Table S3 of the Supplementary Information. Generic removal rates are used to
178 predict emissions since there were no regional of STP-specific removal rates available. It should be
179 noted that the number of inhabitants inhabitant in the Jeker catchment exceeded the inhabitant
180 equivalents of the STPs due to insufficient capacity of the some treatment plants and the fact that not
181 all wastewater is treated in STPs (SPGE, 2006). So the applied generic removal rates in Table 2 might
182 overestimate actual removal of some pharmaceuticals for the Jeker catchment.
183

184 *TABLE 2*

Results and discussion

Analytical results

24 pharmaceuticals and 13 transformation products were observed in the surface waters. Figure 2 shows the concentration ranges of the pharmaceuticals observed and the percentage of positive detections (%) in all samples.

FIGURE 2

The transformation product guanylurea and its parent pharmaceutical metformin show the highest concentrations in surface waters. Furthermore, high concentrations of transformation products such as hydroxy ibuprofen, transdiol carbamazepine, acetaminophen sulphate and desmethyl tramadol are observed. These data show that transformation products can form a relevant proportion of the total pharmaceutical load of surface waters.

Grab sampling

Grab sampling during late autumn might not provide a representative 'average' sample since the emissions of STP show both daily and seasonal fluctuations in pharmaceutical emissions that are related to variations in consumption and treatment performance (ter Laak et al., 2010). The daily variations in pharmaceutical emissions in the Geul and Geleenbeek are likely of minor impact since samples were taken 25 to 34 km downstream of the STP effluents, which is roughly equivalent to 0.5 to 1.0 days residence time (Personal Communication, Harry Tolkamp, Roer en Overmaas Regional Water Authority). The length of the Jeker is 65 km and there are twenty STPs with a total capacity of just over 100,000 inhabitant equivalents along its catchment. Additionally , ten STPs with a total capacity of approximately 35.000 to 40.000 I.E. are planned since various communities are not yet connected to sewage treatment and currently emit untreated wastewater into the Jeker (SPGE, 2006). Hence, for the

211 Geleenbeek, Geul and Jeker, sufficient mixing of the flow rates with relatively pristine river water had
212 occurred before the actual sampling was performed. Contrastingly, the Slijbeek is fed by a single STP
213 located only few km upstream from the sampling location. This sample can therefore be strongly
214 influenced by daily fluctuations of concentrations of pharmaceuticals, so these data should be
215 interpreted with care.
216
217 ***Pharmaceuticals and transformation products in surface waters***
218 Figure 3 shows the ratios of the concentrations of the transformation products (TPs) versus its parent
219 pharmaceutical (PP) on a molar basis for the different rivers. The data of the Geul catchment (n=4) are
220 excluded from the data shown in Figure 3 as concentrations of pharmaceuticals and/or transformation
221 products were often close to limits of quantification, revealing less accurate ratios than for the other
222 sampling locations. Constant ratios enable to estimate concentrations of transformation products from
223 the concentration of the parent molecule or *vice versa*.
224
225 FIGURE 3
226
227 Metformin is excreted unchanged by humans (Van Loenen, 2008). Guanylurea is formed during sewage
228 treatment (Scheurer et al., 2009; Scheurer et al., 2012; Oosterhuis et al., 2013). Likely, similar processes
229 occur in surface waters. However, there are no studies that have illustrated and quantified
230 metformin break down rates in the aqueous environment. There is only sparse circumstantial
231 evidence that suggests that rates in the environment are much lower than in STPs (Oosterhuis
232 et al., 2013). Additional information on environmental degradation of this compound is
233 required to improve predictions on its environmental fate.
234
235 A study of eight Dutch sewage treatment plants revealed that 53 % to 98 % of metformin is removed
236 during treatment (Vergouwen et al., 2011). Furthermore, Oosterhuis et al (2013) reported up to 99 %
237 metformin removal by secondary treatment with a membrane bioreactor and that 91 % and 85% of the

metformin in the influent was recovered as guanylurea in the STP effluent and receiving water, respectively.

The current data show a large variation in the ratio of metformin and guanylurea in surface waters. The average TP/PP ratio for these compounds is 8.7 with a 95 % confidence interval of 1.2 to 16.4 (n=16). Large differences are observed between and within rivers (Figure 3). The TP/PP ratio in the Slijbeek, that is fed by a single Dutch STP varied from 3.4 to 40.1. Similar observations were made in the Geleenbeek that is fed by three Dutch STPs. The samples taken from the river Meuse at Eijsen (Belgian-Dutch border) and Jeker that are fed by Belgian sewage effluents show much lower TP/PP ratios of 0.55 ± 0.28.

For both the Geleenbeek and Slijbeek a clear difference in TP/PP ratio of metformin and guanylurea between the end of dry period (first two samples) and wet period (last two samples) was observed. In the dry period the average TP/PP ratio was 30.1 with a standard deviation of ± 14.8 (n=4) and in the wet period a significantly lower TP/PP ratio of 3.7 with a standard deviation of ± 2.5 (n=4) was observed (T-test, p-value 0.013). The river Meuse and Jeker samples also show significantly different ratios during dry and wet conditions as well (0.77 ± 0.20 and 0.33 ± 0.11 respectively, T-test, p-value 0.007). Possibly rainfall events, that increase flow of wastewater and reduce hydraulic retention times in treatment plants, and slightly lower temperatures during the last two sampling dates (surface water temperature 7.6 to 8.7 °C vs. 8.3 to 10.1 °C) affected the metformin transformation into guanylurea.

This illustrates that the removal of metformin and the formation of guanylurea varies between rivers (or STPs) and with environmental conditions. The limited number of samples and variable ratios do not allow for a reliable estimation of concentrations of the transformation products from analysis of the parent molecule.

For carbamazepine, four human metabolites were observed in the surface waters. Interestingly, the molar ratios of the TPs and PP is similar within and between different surface waters (Figure 3). The TP/PP ratio of 10,11-trandiol carbamazepine is 2.7 with a narrow 95 % confidence interval of 2.6 to 2.9 (n=15). The average TP/PP ratios of the 3-hydroxy carbamazepine, 2-hydroxy carbamazepine and epoxy carbamazepine are 0.20 (95 % CL 0.18 to 0.22, n=15), 0.17 (95 % CL 0.15 to 0.18, n=15) and 0.12 (95 % CL 0.11 to 0.13, n=15), respectively. Figure 4 illustrates the fraction of the consumed carbamazepine that can be retrieved as parent pharmaceutical and the abovementioned transformation products in the surface water of two catchments.

FIGURE 4

Fractions of carbamazepine and transformation products recovered in surface waters appear to be slightly lower but show a strikingly similar pattern to the fractions of these metabolites excreted in urine (Eichelbaum et al., 1985). The excretion of 10,11-transdiol carbamazepine in mono-therapy and therapy combined with other anticonvulsants ranges from 26.5 to 49.9 % of the consumed amount. In this study 20.2 to 31.5 % of the consumed carbamazepine could be recovered as 10,11-transdiol carbamazepine in surface waters. The excretion of 3-hydroxy carbamazepine in urine is 3.0 to 4.2 %, while 1.6 to 2.0 % was recovered surface waters in this study. For 2-hydroxy carbamazepine these values amounted to 2.4 to 3.1 % vs. 1.2 to 2.0 %, and for epoxy carbamazepine 1.1 to 1.5 % vs. 0.8 to 1.3 %.

Renal excretion rates of carbamazepine and its transformation products is well studied (Eichelbaum et al., 1985), but the composition of the fraction that is excreted via feces is (to our knowledge) unknown. In this study 6.3 to 10.3 % of the consumed carbamazepine amount is recovered in receiving surface waters. The recalcitrance of carbamazepine in the sewage treatment and environment (Heberer, 2002) and the stable ratios of the various transformation products that largely correspond to renal excretion

290 suggest that the metabolites are also recalcitrant in sewage treatment and the environment. These
291 constant ratios allow for highly accurate estimation of concentrations of the transformation products
292 from analysis of the carbamazepine and *vice versa*.
293
294 The ratio of tramadol and the human metabolite O-desmethyl tramadol observed in surface waters in
295 the present study is constant. The average TP/PP ratio for these compounds is 0.55 with a 95 %
296 confidence interval of 0.49 to 0.62 (n=14). This is very similar to ratios observed in surface waters of
297 earlier studies (de Jongh et al., 2012) and fall within ratios observed in human urine that range from
298 0.26-0.75 (Lintz et al., 1981; Ardakani Y. H. and R., 2009; Chitil et al., 2009). Apparently, both tramadol
299 and O-desmethyl tramadol are rather recalcitrant and stable ratio is retained in the urban water cycle.
300 This stable ratio enables one to accurately estimate concentrations of O-desmethyl tramadol from
301 analysis of tramadol and *vice versa*.
302
303 The average TP/PP ratio of α-hydroxy metoprolol is 0.15 with a 95 % confidence interval of 0.11 to 0.20
304 (n=11). This ratio is slightly lower than the observed metabolic ratio of 3.1 ± 2.5 that equals a TP/PP
305 ratio of 0.32 (Tu and Zhao, 1995). Minor differences might be explained by either higher removal of α-
306 hydroxy metoprolol during sewage treatment or additional metoprolol excretion via feces that was not
307 accounted for in the cited study. The average TP/PP of sulfamethoxazole and its product N4-acetyl
308 sulfamethoxazole observed in amounts to 0.66 with a 95 % confidence interval of 0.42 to 0.89 (n=15).
309 This TP/PP is one fifth of the reported human excretion ratio of 3.15 (Lienert et al., 2007). Possibly, the
310 acetylated sulfamethoxazole is partially removed or transformed during sewage treatment processes
311 and in surface water. Partial removal or transformation during sewage treatment (Verlicchi et al., 2012)
312 might explain why the TP/PP of metoprolol and sulfamethoxazole and their respective human
313 metabolites show more variation than carbamazepine and tramadol. Unfortunately, there are, to our
314 knowledge, no literature data on STP removal rates of the metabolites to verify this hypothesis.
315 Nevertheless, the TP/PP of sulfamethoxazole and metoprolol appear rather stable and allow to

316 estimate concentrations of the transformation products from analysis of the parent molecule and *vice*
317 *versa* within a factor of two.
318
319 Apparently, the average metabolism of pharmaceuticals by a large population of humans is stable while
320 transformation and sorption processes further down the urban water cycle result in more variable
321 removal rates for some pharmaceuticals. Considering this observation, it is relevant to investigate
322 temperature, oxygen concentration, combination of oxic and anoxic conditions, redox potential,
323 residence time in various treatment processes, composition of the water, applied treatment techniques
324 (composition of) biodegraders in active sludge and adsorption processes on the removal of
325 pharmaceuticals during sewage treatment (Onesios et al., 2009; Pomiès et al., 2013). The TP/PP ratio
326 might be a suitable parameter to monitor such processes and reveal parameters that affect
327 transformation rates. Finally, the potential risks of both parent pharmaceuticals and transformation
328 products need to be assessed for a proper environmental risk assessment.
329
330 ***Loads of pharmaceuticals and transformation products***
331 Loads were calculated for all pharmaceuticals and transformation products that were observed during
332 all four sampling dates.
333
334 FIGURE 5
335
336 The loads are predicted by multiplying the consumed pharmaceuticals by the predicted fraction
337 excreted by the user and the fraction remaining during STP passage (Eq. 3 and Table 2), as discussed in
338 the paragraph “Calculating emissions from monitoring data” above (ter Laak et al., 2010). Figures 5a-c
339 show the observed daily loads of pharmaceuticals versus the predicted loads. Not all observed
340 pharmaceuticals listed in Figure 2 were included since for some of these either Dutch or Belgian sales
341 data were lacking.

342

343 Figures 5a-c show that for some pharmaceuticals the measured loads exceed predicted loads at one or
344 more locations. The largest of these underestimations are observed for the analgesic / anti-
345 inflammatory pharmaceuticals naproxen, ketoprofen and paracetamol. These underestimations are
346 likely explained by additional 'over the counter' sales that are not registered by the Belgian and Dutch
347 monitoring organizations since they are not only sold and documented by official pharmacies nor
348 reimbursed by health insurance. Furthermore, the actual load of the lipid regulator gemfibrozil, is
349 underestimated by a factor three in both the Geleenbeek and Geul . There is no obvious explanation for
350 this underestimation. Gemfibrozil is, however, known to be excreted in conjugated forms (Dix et al.,
351 1999). Potentially these conjugates are de-conjugated during sewage treatment and in the
352 environment, resulting in higher loads of the parent pharmaceutical in receiving waters. Finally,
353 lincomycin, a lincosamide antibiotic, shows a higher concentration in the Jeker than predicted from
354 human consumption. Lincomycin is also used in veterinary practice, so emissions from veterinary use in
355 the Jeker catchment potentially resulted in additional loads in the river.

356

357 Carbamazepine and three antibiotics (sulfamethoxazole, erythromycin A and metronidazole) show a 2
358 to 4 fold lower concentration than predicted from sales. The overestimation of the concentrations of
359 the antibiotics might be related to uncertainties in the reported removal rates by sewage treatment or
360 metabolism, since the removal in these to processes is both relevant (Table 2). The consistent factor
361 overestimation of carbamazepine concentrations cannot be explained by uncertainties in reported
362 removal rates because carbamazepine is very persistent in sewage treatment and the environment
363 (Clara et al., 2004). Oosterhuis (2013) monitored carbamazepine concentrations in sewage influent,
364 effluent and receiving surface waters and compared these to their local sales data. A similar
365 overestimation was observed in this study, (Oosterhuis et al., 2013). The predicted environmental loads
366 calculated in both studies are based upon excretion rates obtained from Lienert et al (Lienert et al.,
367 2007). Lienert justly discusses the relevance of fecal excretion of carbamazepine for emissions into the

368 environment, a factor that is often neglected in pharmacological studies. There is, however, no data
369 supporting the assumption that the fecal excretion is solely as parent pharmaceutical. The observed
370 loads in the surface waters and ratio with transformation products in the present study suggest that the
371 total excretion of non-metabolized carbamazepine is rather around 10 % than the 26 % proposed by
372 Lienert et al. or the 1 to 3 % found in urine (Lienert et al., 2007). However, additional studies are
373 required to test this hypothesis.
374
375 In this study the discrepancies between observed and predicted loads are a result of analytical
376 uncertainties (small), uncertainties introduced by grab sampling (variable, depends on sampling
377 location and sampling date and time), regional variation in consumption (generally small within
378 countries, larger between countries), seasonal variations in consumption (relevant for some
379 pharmaceuticals), uncertainties in sales data (relevant for some pharmaceuticals), transformation and
380 excretion rates (relevant for some pharmaceuticals, fecal excretion is generally studied less) variations
381 in removal efficiencies of sewage treatment (can strongly vary within and between treatment plants).
382 Discrepancies in the order of a factor three are therefore likely to be observed (Alder et al., 2010; ter
383 Laak et al., 2010). Local data on consumption, excretion and treatment specific removal rates as well as
384 improved sampling during different seasons can increase the accuracy of the prediction to some extent,
385 but a certain degree of uncertainty will remain.
386
387 **Per capita emission of pharmaceuticals and transformation products**
388 The loads of the pharmaceuticals and transformation products present in all datasets are normalized
389 per inhabitant and presented in Figure 6. The data illustrate that the per capita load of pharmaceuticals
390 is higher for the Geul and Jeker compared to the Geleenbeek catchment. The per capita loads of the
391 Slijbeek were not calculated since there were no flow rate data available. The loads in the river Meuse
392 were not calculated because loads can't be directly linked to sales, since this is a complex water system

with variable flow rates between the river Meuse and various canals that are fed by the river Meuse and circumvent the sampling location at the Belgian-Dutch border.

FIGURE 6

Comparing per capita emissions from Belgium and the Netherlands

The Geul and Jeker showed a significantly higher per capita load of pharmaceuticals and transformation products than Geleenbeek (Figure 6). The Geul and Jeker (also) transport effluents from Belgian origin. This suggests that more pharmaceuticals are used in Belgium and/or that removal by sewage treatment in the Belgian part of the catchment is less efficient or not present (SPGE, 2006). The Geleenbeek and Jeker were selected for a more detailed comparison, since the Geul is fed by both Dutch and Belgian sewage effluents which might result in ambiguous data.

FIGURE 7

In Figure 7 per capita loads of the Belgian (Jeker) and Dutch (Geleenbeek) populations are plotted against each other. Since most of the data points in Figure 7 are situated at the right hand side of the 1:1 line (diagonal in Figure 7), most pharmaceuticals and transformation products show a higher *per capita* load from the Belgian population. This corresponds to higher total *per capita* loads shown in Figure 6. Only a few pharmaceuticals and transformation products show higher *per capita* loads from the Dutch population. Part of the differences in *per capita* loads can be explained by differences in pharmaceutical consumption in Belgium and the Netherlands. For example, Dutch per capita sales of metoprolol are eight times higher than Belgian sales (SFK & RIZIV). This is reflected in the higher *per capita* load of both metoprolol and α-hydroxy metoprolol in the Geleenbeek. Furthermore, the two fold higher *per capita* load of furosemide in the Geleenbeek compared to the Jeker also corresponds to differences in sales in the Netherlands and Belgium (SFK & RIZIV). Finally, the absence of the lipid

419 regulator gemfibrozil in waters originating from Belgium (Meuse and Jeker) can be explained by the fact
420 that it is not prescribed and reimbursed in Belgium (Belgian Centre for Farmacotherapeutic Information
421 CFI, 2014).
422
423 On the other hand, the *per capita* loads of metformin, tramadol and its de-methylated transformation
424 product, venlafaxine, naproxen, paracetamol, propranolol, ketoprofen and metronidazole exceed the
425 Dutch *per capita* load over a factor three. For propranolol, tramadol and venlafaxine, the higher *per*
426 *capita* loads can be explained by higher consumption in Belgium. Contrastingly, the consumption of
427 metformin is very similar in both countries (SFK en RIZIV). Probably the higher *per capita* load of
428 metformin is explained by absence or less efficient of treatment of part of the Belgian sewage. This
429 hypothesis is supported by the lower formation of guanylurea/metformin ratioin waters from Belgium
430 (see paragraph "Pharmaceuticals and transformation products in surface waters"). The higher *per*
431 *capita* loads for the analgesics and anti-inflammatory pharmaceuticals ketoprofen, naproxen,
432 paracetamol and ibuprofen (hydroxy ibuprofen is detected) are more difficult to explain from sales data
433 since part of these pharmaceuticals are sold 'over the counter' without being registered.
434
435 **Implications**
436 The current study illustrates that there are regional differences in pharmaceutical loads and
437 concentrations in different catchments can be related to differences in sales patterns and absence of
438 sewage treatment. A better understanding of the relation between consumption, excretion processes
439 and removal or transformation efficiencies of STPs, emissions and further dilution, sorption and
440 transformation in rivers and river systems enables to optimize use of (limited) monitoring activities. For
441 example, relations between the occurrence of pharmaceuticals and transformation products and
442 potentially even between different pharmaceuticals or between sales data and occurrence data can
443 extend the application of monitoring data. Furthermore, knowledge on transformation processes and

444 transformation products generated in sewage treatment also enables one to monitor and potentially
445 interpret sewage treatment efficiency and further transformation in surface waters.
446
447 **Conclusions**
448 • Transformation products represent a relevant fraction of the total concentration of
449 pharmaceuticals in surface waters
450 • Stable ratios between pharmaceuticals and their transformation products allow estimation of
451 concentrations from one to the other
452 • Variable ratios of metformin and guanylurea might be applied as an indicator of sewage
453 treatment performance and discharge of untreated sewage
454 • Sales data, excretion from the body and removal in STPs can be used to estimate loads in
455 surface waters for most pharmaceuticals
456 • per capita loads of most pharmaceuticals from Belgium are significantly higher than those from
457 the Netherlands
458 • Information on (regional) sales or consumption of pharmaceuticals and transformation
459 products can provide a better qualitative and quantitative understanding of their occurrence in
460 surface waters
461
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Figure captions

Figure 1: Sampling locations. Slijbeek (1), Geleenbeek (2), Geul (3), Jeker (4) and Meuse at the Belgian-Dutch border (5)

Figure 2: Box/whisker plots of the concentration ranges and detection frequency of pharmaceuticals and transformation products in all surface water samples (n=20). The boxes represent the 25 to 75 percentile with the median value indicated in the center, the whiskers give maximum and minimum values.

Figure 3: Box/whisker plots of the molar ratio between concentration of transformation products (TP) and their parent pharmaceutical (PP) in surface waters (TP/PP). For each location four samples were used. The exceptions are; carbamazepine ratios in de Meuse (n=3), tramadol ratios in the Slijbeek and Meuse (n=3), metoprolol ratios in the Jeker (n=3) and Meuse (n=0) and sulfamethoxazole ratios in the Slijbeek (n=3). The boxes represent median and 25 and 75% and the whiskers give maximum and minimum values.
The following abbreviations are used in the figure: MET = metformin, GA = guanylurea, CBZ = carbamazepine, 10,11-DiOH CBZ = 10,11-trans diol carbamazepine, 3-OH CBZ = 3-hydroxy carbamazepine, 2-OH CBZ = 2-hydroxy carbamazepine, epoxy CBZ = epoxy carbamazepine, SMZ = sulfamethoxazole, N4-AC SMZ = N4-acetyl sulfamethoxazole , TRM = tramadol, O-DM TRM = O-desmethyl tramadol, MTL = metoprolol and α-OH MTL = α-hydroxy metoprolol.

Figure 4: Fraction of the sold carbamazepine retrieved in surface water of the Jeker and Geleenbeek

Figure 5a-c: Measured vs. predicted loads of pharmaceuticals for the Geleenbeek (a) Jeker (b) and Geul (c) catchment. The solid line represents the 1:1 relation and the dashed lines are the 1:3 and 3:1 relation of measured and predicted loads. The error bars represent standard deviations

Figure 6: Box/whisker plots of the daily load per inhabitant with (A) and without (B) metformin and guanlyurea. It should be noted that metformin and its transformation product account for 53 to 79 % of the total load in the abovementioned rivers. The figure only depicts the pharmaceuticals that were observed in 100 % of all samples of the tributaries. The broken lines indicate statistical differences (T-test *p*-value <0.01) between the values of the Geleenbeek and the Geul and the Geleenbeek and the Jeker. No statistical differences between Geul and Jeker were observed (*p*-value > 0.05).

Figure 7: Per capita loads of pharmaceuticals, the Geleenbeek from the Netherlands vs. the Jeker from Belgium. The solid line represents the 1:1 relation and the dashed lines are the 1:3 and 3:1 relation of measured and predicted loads. The error bars represent standard deviations

literature of figures, do not include these lines in the manuscript, it is solely used to correctly update the literature list using Endnote

(SPGE, 2006; Van Loenen, 2008; RIWA Maas, 2009) (Carr et al., 1992) (Lienert et al., 2007) (Ardakani Y. H. and R., 2009) (Prescott, 1980) (Howell et al., 1993) (European Medicines Agency, 1998) (Lamp et al., 1999) (Vergouwen et al., 2011) (Wick et al., 2009) (Castiglioni et al., 2006) (Radjenovic et al., 2007) (Rúa-Gómez and Püttmann, 2012) (Radjenovic et al., 2007) (Miege et al., 2009) (Watkinson et al., 2007) (Rosal et al., 2010) (European Medicines Agency, 1997; Grubb et al., 1999)

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